The first minutes of reperfusion: a window of opportunity for cardioprotection

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Abstract

During the past decade, the understanding has grown that control of the conditions of reperfusion is critical for salvaging ischemic–reperfused myocardium. The first few minutes of reperfusion constitute a critical phase, as here lethal tissue injury in addition to that already developed during ischemia may be initiated. The identification of the mechanisms of reperfusion-induced cell death opens a new window of opportunity for cardioprotection in the clinic. Development of cardiomyocyte hypercontracture is a predominant feature of reperfusion injury. We and others have shown that control of hypercontracture in reperfusion reduces the extent of tissue injury. On the cellular level, it was shown that reperfusion-induced hypercontracture might either originate from a rigor-type mechanism, when energy recovery proceeds very slowly, or from Ca\(^{2+}\) overload, when energy recovery is rapid but cytosolic Ca\(^{2+}\) load is high. These two mechanisms can be influenced by various interventions that either connect with cytosolic Ca\(^{2+}\) control or myofibrillar Ca\(^{2+}\) sensitivity or with mitochondrial energy production. These experimental approaches will hopefully lead to novel strategies for clinical cardioprotection during the early phase of reperfusion.

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1. Introduction

In spite of its eminent clinical importance, our understanding of the causal mechanisms of myocardial ischemia–reperfusion injury is still incomplete. One of the great achievements of clinical cardiology is the introduction of therapeutic interventions that allow early reperfusion of the ischemic myocardium. These new therapeutic possibilities gave rise to the question of whether modification of reperfusion conditions can reduce the extent of post-ischemic myocardial necrosis, but none of these experimental strategies have, as yet, found their way into clinical practice. If it is possible to limit infarct size with an intervention at the time of reperfusion, a part of the injury developing without such an intervention in ischemic–reperfused hearts must be due to causes originating within the reperfusion period. Injury produced by such causes has been termed “reperfusion injury” or, more specifically, “lethal reperfusion injury” if one refers to causes leading to cell death and therefore infarct size enlargement. Reperfusion injury of the myocardium is a complex phenomenon consisting of several independent etiologies. During the earliest phase (minutes) of reperfusion, development of cardiomyocyte hypercontracture seems to be the primary cause of cardiomyocyte necrosis. Thereafter, lasting for hours, various additional causes can lead to further increase of cell death either by necrosis or apoptosis, and vascular failure may further aggravate cardiomyocyte injury. This temporal sequence of pathological events has implications for potential therapeutic approaches to reperfusion injury. What comes first must be treated first, as otherwise the opportunity for specific treatment is lost. Therefore, in this brief review we would like to draw attention to the possible
therapeutic interventions during the earliest phase of reperfusion. We will concentrate on mechanisms leading to the death of myocardial cells, i.e. causes for severe reperfusion injury, as opposed to temporary functional impairment.

Cardiac surgeons have long observed rapidly developing lethal reperfusion injury. In cardiac surgery, when hearts are reperfused after prolonged ischemia and or unsatisfactory intra-operative cardioplegia, reperfusion occasionally provokes the “stone heart” phenomenon, i.e. a stiff and pale heart resulting from massive muscle contracture and the concomitant loss of cellular protein content. This stone heart phenomenon belongs to the early phase of reperfusion, as it develops within minutes. Histologically, stone hearts present hypercontracted myofibrils and ruptured cellular membranes. A similar pattern of contracture and necrotic cell injury, termed “contraction band necrosis”, is also observed after regional ischemia and reperfusion in myocardium [1]. The histological picture is characterized by the existence of super-contracted sarcomeres and sarcolemmal disruption. It can be explained as a result of strong and inhomogeneous mechanical forces. In the heart in situ exposed to transient coronary occlusion the area of necrosis is composed almost exclusively of contraction band necrosis [2,3]. The extent of contraction band necrosis correlates well with the magnitude of macroscopic myocardial shrinkage during the first minutes of reperfusion, and with the magnitude of enzyme release occurring during the initial minutes of reflow [4]. These observations indicate that post-ischemic necrosis and development of hypercontracture are causally related phenomena. Experimental studies have indeed demonstrated that it is the development of contracture that triggers reperfusion-induced necrosis. The key experiments were performed more than a decade ago [3,5]. These experiments, which involved either isolated cardiomyocytes or the whole heart in situ, demonstrated that a temporary contractile blockade of reperfused myocardium, applied for the first few minutes of reperfusion, is sufficient to reduce the extent of developing cell injury and infarct size. These original observations made it clear that the understanding of reperfusion-induced hypercontracture is of key importance for the understanding of lethal reperfusion injury originating from the first minutes of reperfusion.

2. Two causes for reoxygenation-induced contracture

Contracture, i.e. a sustained shortening and stiffening of the myocardium, can have several causes. In ischemic myocardium, contracture develops by a rigor-type mechanism. Studies on skinned cardiac cells or muscle fibers have shown that a force, generated by slow cross-bridge cycling, is initiated when cytosolic ATP is reduced to a low (<100 μM) but non-zero level [6,7]. In ischemia, this window of low cytosolic ATP concentrations is open only during a brief period of time since cellular ATP reserves are quickly exhausted. Thereafter, the myofibrillar shortening stays fixed as all cross-bridges between actin and myosin remain in an attached state. The contracture developed by this
ischemic mechanism is moderate in its extent and does not actually cause major structural damage but it leads to cytoskeletal defects. These defects render cardiomyocytes more fragile and thus susceptible to mechanical damage [8]. When energy depletion is rapidly relieved, as by an early onset of reperfusion, ischemic rigor contracture is usually found to be reversible.

Reperfusion-induced hypercontracture observed in reperfused myocardium after prolonged ischemia is characterized by a much greater myofibrillar shortening and cytoskeletal damage as compared to myocardium in ischemic rigor contracture. In terms of ventricular function, the development of this aggravated form of contracture leads to a marked rise in end-diastolic pressure and ventricular wall stiffness during the first minutes of reperfusion. In a number of studies, we have shown that the pathogenesis of reperfusion-induced hypercontracture can be analyzed on the cellular level. This analysis has revealed two independent causes of reperfusion-induced hypercontracture: Ca$^{2+}$ overload-induced contracture and rigor-type contracture (Fig. 1). Calcium overload-induced contracture is elicited in a cardiomyocyte if it develops Ca$^{2+}$ overload during ischemia and is then rapidly re-energized. High cytosolic [Ca$^{2+}$] plus energy leads to uncontrolled activation of the contractile machinery. Rigor-type contracture may be activated during reoxygenation if re-energizing of the ischemic cardiomyocytes occurs at a very low rate. Therefore, it may be observed after prolonged or severe ischemia. Rigor-type contracture is not necessarily dependent on Ca$^{2+}$ overload. These two causal mechanisms for reperfusion-induced hypercontracture are described separately as follows.

3. Ca$^{2+}$ overload-induced contracture

Ischemic cardiac cells become energy depleted and subsequently develop a Ca$^{2+}$ overload of the cytosol due to an initial accumulation of Na$^+$ and a subsequent uptake of Ca$^{2+}$ through a reverse-mode operation of the sarcolemmal Na$^+$/Ca$^{2+}$ exchanger (Fig. 2). At the end of ischemia, this leaves the ischemic cardiomyocytes in a state of cytosolic Ca$^{2+}$ overload. If the ability of mitochondria to resume ATP synthesis is not critically impaired during the ischemic period, reoxygenation brought on by reperfusion leads to a rapid recovery of oxidative energy production. Resynthesis of ATP can enable cardiomyocytes to recover from the loss of cytosolic cation balance but it also reactivates the contractile machinery. Contractile activation is normally faster than Ca$^{2+}$ recovery and this leads to an uncontrolled Ca$^{2+}$-dependent contraction. When analyzed in detail, it was found that cyclic uptake and release of Ca$^{2+}$ by the sarcoplasmic reticulum (SR) in the reoxyg enated cardiomyocytes immediately triggers a Ca$^{2+}$ overload-induced hypercontracture [9,10] (Fig. 3). These oscillatory Ca$^{2+}$...
shifts lead to high cytosolic peak Ca$^{2+}$ concentrations. The frequency of these Ca$^{2+}$ peaks is influenced by an ongoing Ca$^{2+}$ influx across the sarcolemma during the early phase of reoxygenation [8]. During this period, the transsarcolemmal Na$^+$ gradient is still reduced and the Na$^+$/Ca$^{2+}$ exchanger still operates in the reverse mode. Experimentally, various protocols have been shown to interfere with Ca$^{2+}$ overload-induced contracture. First, contracture can be prevented by an initial, time-limited inhibition of the contractile machinery. For this purpose, the chemical phosphatase 2,3-butanedione monoxime has been used [3,5]. Part of the protective effects of cGMP-mediated effectors (NO, ANP) or cytosolic acidosis can also be attributed to contractile inhibition, as these agents reduce the Ca$^{2+}$ sensitivity of myofibrils. Second, reducing SR-dependent Ca$^{2+}$ oscillations can reduce contracture. This can either be achieved by agents interfering with SR Ca$^{2+}$ sequestration or by inhibition of the Ca$^{2+}$ influx into the cells still occurring during the early phase of reoxygenation. Ca$^{2+}$ cycling across the SR can be inhibited by specific agents interfering with SR Ca$^{2+}$ ATPase or SR Ca$^{2+}$ release [9] or with less specific means such as the anesthetic halothane or intracellular acidosis [11,12].

Of particular interest is the therapeutic value of proton transport inhibition during the early phase of reperfusion. We showed that preservation of ischemic intracellular acidosis during the initial phase of reperfusion protects cardiac cells against reoxygenation-induced hypercontracture [12]. To achieve effective protection, simultaneous inhibition of two acid extruder mechanisms is required, i.e. the Na$^+$/H$^+$ exchanger (NHE) and the Na$^+$/HCO$_3^-$ symporter (NBS). Sole applications of NHE inhibitors have failed to provide myocardial protection during reperfusion, both in experimental [13,14] and clinical studies [15]. When cardiomyocytes are reoxygenated in the presence of constant cytosolic acidosis, they can recover metabolically while contractile activation remains inhibited and SR Ca$^{2+}$ movements are attenuated. Metabolic recovery drives the re-normalization of cellular cation control, thus removing the causes for Ca$^{2+}$-induced contracture.

4. Reoxygenation-induced rigor contracture

As long as mitochondrial energy production recovers rapidly upon reperfusion, reoxygenated cardiomyocytes are in acute jeopardy of Ca$^{2+}$ overload-induced contracture. After prolonged ischemia, however, this mechanism of contracture development is less and less likely to occur. With progression of ischemic cellular damage, the ability of mitochondria to rapidly restore a normal cellular state of energy upon reoxygenation is reduced. During the early phase of reoxygenation, cardiomyocytes may thus contain very low (although rising) concentrations of ATP that provoke rigor contracture (see above). In comparison to ischemia, reoxygenated cardiomyocytes may spend much more time within the window of low cytosolic ATP levels suitable to induce rigor-type contracture. Therefore, rigor-type cell shortening can be much more pronounced than observed in ischemic rigor contracture. In fact, the rigor mechanism may become the major contributor to reoxygenation-induced hypercontracture [16]. In cases where rigor
contracture prevails in acute reperfusion injury, therapeutic actions aimed at cytosolic Ca\(^{2+}\) overload are not effective as rigor contracture is essentially Ca\(^{2+}\)-independent. It can be shown experimentally that rigor contracture can be reduced at the time of reperfusion by improving the conditions for energy recovery. A first approach is the application of mitochondrial energy substrates, e.g. succinate, with the aim of accelerating oxidative energy production. Secondly, one may speculate about a means to protect mitochondria from compulsory calcium uptake, as this allows them to resume oxidative phosphorylation more rapidly during the early phase of reperfusion.

5. Spread of hypercontracture

The cellular mechanisms that contribute to reperfusion-induced hypercontracture seem to represent the major causes for lethal cell injury occurring during the early phase of reperfusion. Model calculations have suggested, however, that they cannot explain the continuous geometry of contraction band necrosis in reperfused myocardium [17]. Cell–cell interactions seem to take part in the expansion of early necrosis. Recent studies have shown that gap junction-mediated communication between ischemic cells allows spreading of cell injury during myocardial reperfusion [18]. The passage of sodium through gap junctions from hypercontracting cells to adjacent cells and the subsequent change in cytosolic Ca\(^{2+}\) levels through reverse-mode Na\(^+/\)Ca\(^{2+}\) exchange may result in propagation of contracture [19]. It seems also possible that metabolic coupling synchronizes the rate of ATP recovery in reperfused myocardium and therefore equalizes the development of rigor contracture. Apart from these chemical coupling mechanisms, cells undergoing contracture exchange forces with their neighbors. This may cause mutual mechanical damage and contribute to the spread of necrosis.

6. Protective signaling pathways during the early phase of reperfusion

Investigation of the underlying causes of cardiac protection provided by ischemic preconditioning has led to the identification of protective cellular signaling mechanisms. Some of these mechanisms have also been found to provide protection by modifying not the conditions of ischemia but those of reperfusion. An example is given in our own previous work in which we imitated the protective role of ischemic preconditioning by a pharmacological pre-ischemic activation of protein kinase C [20]. We found that this treatment did not improve the cellular state of energy or the progressive loss of control of cation homeostasis during ischemic conditions, but it markedly attenuated the development of reoxygenation-induced hypercontracture. This observation indicated that the preconditioning protection might be caused by an altered reperfusion scenario. Recent studies by Yellon’s group in ischemic–reperfused heart

![Fig. 4. Scheme of proposed mechanism of PKG-mediated reperfusion protection. Activation of particulate or soluble guanylyl cyclase (pGC, sGC) leads to activation of PKG. Phosphorylation of phospholamban (PL) causes enhanced Ca\(^{2+}\) uptake by the SR through activation of the SR Ca\(^{2+}\) pump (SERCA), thereby augmenting the internal Ca\(^{2+}\) sequestration. Phosphorylation of troponin I (TnI) causes a reduced Ca\(^{2+}\) sensitivity of the myofibrils. Both effects reduce the risk of Ca\(^{2+}\)-overload contracture in reoxygenated cardiomyocytes.](https://academic.oup.com/cardiovascres/article-abstract/61/3/365/401250)
showed that a preconditioning protocol could be imitated in its protective potency in the same signaling pathways activated at the time of reperfusion [21,22]. These experiments confirm that, at least under certain circumstances, preconditioning acts through a change in the conditions of reperfusion.

Studies on preconditioning and other work have directed interest towards the role of defined signaling pathways that may confer protection in reperfused myocardium. The protective effects of protein kinase G-dependent signaling and PI 3-kinase signaling seem to be firmly identified. The first signaling pathway can be activated by NO donors [23–25] or members of the natriuretic peptide family [26–28], the latter, e.g., by insulin [29]. Stimuli of the PKG-dependent pathway can interact with several players in the scenario of reperfusion-induced hypercontracture development (Fig. 4). The action of PKG on myofibrils (potential target: troponin I) causes a Ca$^{2+}$ desensitization that is beneficial in reoxygenated cells overloaded with Ca$^{2+}$. Its action on SR Ca$^{2+}$ ATPase (potential target: phospholamban) inhibits SR-dependent Ca$^{2+}$ cycling in reoxygenated cardiomyocytes [28]. Both actions attenuate Ca$^{2+}$-induced contracture development. Using NO donors in reperfused myocardium also has other beneficial effects such as leukocyte inhibition and vasodilatation. It should be noted, however, that NO donors might also induce apoptosis in cardiomyocytes by either a free radical-mediated mechanism or via cGMP signaling [30].

Recently, by Sack and Yellon [29] demonstrated that insulin applied at the time of reperfusion provides myocardial protection against impending cell death through a PI 3-kinase-mediated pathway. The authors have attributed this protective effect to an anti-apoptotic action of this pathway. Their conclusion is based on the observation that under this treatment certain parameters of apoptotic cell death are less frequent in reperfused myocardium. However, since the quantification of these parameters is notoriously uncertain, it remains unclear whether this indeed indicates a causal role. Recent studies from our group have shown that insulin applied to isolated cardiomyocytes under reperfusion conditions protects the cells against reperfusion-induced hypercontracture and therefore against an underlying cause for acute reperfusion-induced necrosis [31]. We found that under these conditions the occurrence of reperfusion-induced oscillations of cytosolic [Ca$^{2+}$] is greatly reduced, similar to other interventions that provide protection during the early phase of reperfusion. Therefore, it seems possible that the newly identified PI 3-kinase-dependent pathway of protection against reperfusion injury works primarily via a reduction of hypercontracture-induced cellular necrosis.

7. Conclusion

The early phase of reperfusion represents an important target for strategies protecting ischemic-reperfused myocardium. These strategies may be aimed primarily at (i) the changes in cellular Ca$^{2+}$ homeostasis, specifically at the rapid Ca$^{2+}$ oscillations, (ii) the control of pH in the reoxygenated cell, or (iii) signaling pathways that influence Ca$^{2+}$ homeostasis or myofibrillar Ca$^{2+}$ sensitivity. Any of these strategies can reduce the risk of myocardial hypercontracture development during the first minutes of reperfusion, which seems to be the main mechanism of an early reperfusion-induced cell death. Adaptation of these protective strategies to clinical therapeutic use would represent a major advancement in the treatment of acute myocardial infarction and in myocardial protection during cardiac surgery.

References


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